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Award Number: DAMD17-00-1-0523

TITLE: Genetic Susceptibility to Prostate Cancer in the Netherlands

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REPORT DATE: August 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	Aug 2003	Annual (1 Aug 2002 - 31 Jul 2003)

4. TITLE AND SUBTITLE
Genetic Susceptibility to Prostate Cancer in the Netherlands

5. FUNDING NUMBERS
DAMD17-00-1-0523

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Studies of the genetics of populations that were historically endogamous for reasons of geography, religion, or language have contributed substantially to our understanding about hereditary predisposition to cancer. One such population is found in the Netherlands where multiple, prevalent, population-specific founder mutations have been identified. Genetic susceptibility to prostate cancer is being investigated in the Netherlands Cohorts Study (NLCS) on Diet and Cancer, which has identified over 800 cases of prostate cancer among 58,279 male participants. From this study, we have identified 300 cases and 300 controls from among men of comparable age to identify markers near prostate cancer susceptibility genes that are present at higher frequency in the group of men with prostate cancer. We have optimized the methods for analysis of these markers. During the next year, we will apply these methods to analyzing the samples that have been collected.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate cancer, linkage disequilibrium, association	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). The Dutch represent such a population.

BODY

The project started six months late in the Netherlands because of delays in contract negotiation. An additional 6-month delay occurred as the result of the terrorist events in New York City of 11 September 2001, the recruitment of qualified technical personnel to perform the work both in New York and in the Netherlands (previously noted in a letter for a no-cost extension) and from receiving samples from Dutch pathology laboratories. A timetable for the statement of work and the accomplishments for each of the tasks follows.

Task 1A. Subject identification and selection. Months 6-20 (completed)

The collection and extraction of samples is now complete and available for analysis. The medical histories of each of these subjects were reviewed, confirming diagnosis of prostate cancer, and noting age and TMN classification at time of diagnosis. Tissue blocks were obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized to provide 50-fold amplification, then applied to the samples.

At the time that the proposal was submitted, we postulated that 800 cases would be available at the time that we initiated this study. This is based on the projection that 8.3 years of follow-up information. In fact, 7.3 years of follow-up information was available and 704 (not 641) cases were identified. From these, we collected samples for 498 cases from the Dutch pathology laboratories. We then selected the 300 samples with the certain normal tissues. This meets our goal of generating 160 genotypes per case, or 48,000 genotypes from all of the cases (half the 96,000 budgeted in the proposal).

Task 1B. Control identification and selection. Months 6-20 (completed)

Buccal swab samples were collected from the whole subcohort of the Netherlands Cohort Study on Diet and Cancer and DNA was extracted from these samples. This included buccal swabs from 940 males. From these, 300 controls with the highest yields of DNA were selected for PEP amplification and genotyping. These samples have been found to be representative of the whole NLCS. Based on generating 160 genotypes per sample, 48,000 genotypes will be generated from controls (half the 96,000 budgeted in the proposal).

Task 2A. Markers from regions associated with loss of heterozygosity in prostate cancer not currently available in the laboratory will be identified and fluorochrome-labeled primers will be synthesized. Months 1-12 (completed)

We identified microsatellite markers for each of the following chromosomal regions 1q24-q25, 7q31, 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, Xq11-q13. Because of uncertainties about relative map positions, we confined our markers to those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (http://www.ncbi.nlm.nih.gov/GeneMap99) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998).

Task 2B. Standard PCR conditions will be developed for each of these markers. Months 1-12 (completed)

The primer sequences for each of these markers was identified using standard databases (http://www.gdb.org). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized. Different thermostable enzymes were tested for their fidelity for amplifying microsatellites, including AmpliTaq, AmpliTaq Gold, Platinum Taq, Platinum Tsp, and Expand High Fidelity. Among these enzymes, Platinum Tsp (Life Technologies, Gaithersburg, MD) was found to produce the most reliable amplification with the least stutter and the least random addition of an adenine at the 3' end of the PCR product. For each of the markers, different PCR conditions were tested, varying temperature and magnesium chloride concentrations, and the optimum conditions were defined.

Task 2C. Individuals with alleles of known sizes will be identified for use in subsequent genotyping analyses. Months 1-12 (completed)

Individuals with alleles of known sizes will be identified for use in subsequent genotyping analyses.

Task 2D. none indicated in original proposal.

Task 3A. Genotype analysis of each marker will be performed for each individual. Months 36-46 (underway)

Genotyping for each individual in the study is being performed for each of the markers. Allele calling is performed using the ABI genotyper software. Whenever possible, genotypes for multiple markers, each tagged with a different fluorochrome are run in the same lane to conserve on the number of electrophoretic runs. Each lane contains internal controls for calibration. Samples with alleles of known size are included in each run to validate the calibration. In addition, one or more samples are typed in duplicate in each run to assess the reproducibility of results. Each of the microsatellites is called independently by at least two individuals and entered into a spreadsheet. Should the results be discordant, then they meet to attempt to resolve these discordances. Should that not be possible, then the analyses are repeated.

Task 3B. Statistical analysis will be performed. Months 36-46 (underway)

Standard calculations of odds ratios using contingency tables and logistic regression analysis are being performed in real time as data for cases and controls are generated to estimate the risks associated with each marker.

Task 4A. To assure the quality of the data, a sample will be reanalyzed and repeat genotyping will be performed, if indicated. (not yet done)

Task 4B. A final report and initial manuscripts will be prepared. (not yet done)

KEY RESEARCH ACCOMPLISHMENTS

Development of DNA databases from cases and controls for genomic analysis.

Completed collection, DNA extraction, and selection of samples for this study.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded by the National Cancer Institute.

Rene Vogels Award, Dutch Society of Oncology to Maurice Zeegers, Ph.D., co-investigator, for research on genetic susceptibility to prostate cander.

CONCLUSIONS

This works demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

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